stimuli is the acoustic vibration mechanically exciting the entire body, but differentiates for each type of tissue, structure or system. Considering that the acoustic stimuli are nonlinear and the body exposure to acoustic field is differentiate on report of time, density of energy and structural acoustic impedance of tissues, than a more realistic human exposure to acoustic stimuli model could be obtained. This paper, consistent with the Fröhlich theory [1] aims to define a Molecular Dynamics (MD) model of Na^+, K^+-ATPase designed to predict not only the coherent elastoelectric oscillations of electric polar cellular structures but the influence of the environmental stimuli (acoustic and thermal vibrations) as well. The transducer role is played by the excitable amino acid chains of the proteins from the ion channels. Their density of energy is continuously changing according to the weak vibrations and rotations of these basic live modules during the harvesting energy process. The MD model and the *in vivo* and *in vitro* validation experiments [2] reveal quantifiable similarities between the periodic characteristics of voltage activation of ionic pumps (Na^+–K^+) through ion channels and the periodic acoustic wave propagation throughout the cells. The rhythm mimetic behavior of heart rate to nonlinear acoustic stimuli (music) is largely known, and this study reveals that it may actually originate within the cellular and neuronal mecanoelectric transduction.

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doi:10.1016/j.bbabio.2014.05.266

S10.P12

Antagonists of tubulin–VDAC interaction induce oxidative stress and mitochondrial dysfunction

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BACKGROUND: Mitochondrial oxidative phosphorylation, membrane potential ($\Delta\Psi$) formation and generation of reactive oxygen species (ROS) require flux of metabolites into mitochondria through voltage dependent anion channels (VDAC). Free tubulin reversibly blocks VDAC both *in vitro* and in cells. Erastin, a small molecule lethal to cancer cells, antagonizes blockade of VDAC by tubulin and upregulates mitochondrial metabolism. We hypothesized that erastin and related "erastin-like" compounds open VDAC, increase mitochondrial metabolism and ROS formation, and activate JNK, which in turn cause mitochondrial dysfunction and cell death. Our AIM was to evaluate the effects of erastin/erastin-like compounds on $\Delta\Psi$, NAD(P)H, ROS, JNK and cell killing.

METHODS: Using confocal fluorescence microscopy, $\Delta\Psi$ was assessed with tetramethylrhodamine methylester (TMRM) and ROS with MitoSOX Red and chloromethyldichlorofluorescein (cmDCF). Autofluorescence of mitochondrial NAD(P)H was assessed by multiphoton microscopy. Total and phosphorylated JNK was determined by immunoblotting. Cell death was monitored by propidium iodide fluorometry

RESULTS: In lipid bilayers, erastin reversed and prevented tubulin inhibition of VDAC. In HepG2 human hepatocarcinoma cells, erastin increased $\Delta\Psi$ by 46% and NAD(P)H by 30%, beginning within 30 min. Subsequently, mitochondria depolarized (3–4 h), indicating mitochondrial dysfunction. Erastin-like compounds X1 and X2 were identified in a high-throughput screening and similarly caused mitochondrial hyperpolarization/depolarization. As mitochondria hyperpolarized, ROS formation increased, which was then followed by mitochondrial depolarization and cell death. In addition, erastin activated JNK (maximal pJNK at 60 min). JNK activation and ROS formation both preceded mitochondrial depolarization and cell death.

CONCLUSION: Erastin and erastin-like compounds reverse tubulin-dependent inhibition of VDAC conductance, leading to mitochondrial hyperpolarization, increased ROS production and activation of the stress kinase JNK. These events appear to induce mitochondrial dysfunction, onset of the mitochondrial permeability transition, and ultimately cell death.

doi:10.1016/j.bbabio.2014.05.267

S10.P13

The discovery of functionally diverse membrane pyrophosphatase subfamilies

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Membrane pyrophosphatases (mPPases) transport H^+ and Na^+ through membranes by harnessing the energy of pyrophosphate hydrolysis, thus creating gradients of these ions across membranes that enable ATP synthesis and secondary transport. Predominantly α-helical mPPase homodimers, formed from ~75 kDa monomers, reside in the membranes of plants, bacteria, archaebacteria, and protists [1]. Until the discovery of three Na^+-transporting PPases in 2007 [2], mPPases had generally been thought to be H^+-transporters. To elucidate the previously obscure functional versatility of mPPases, we selected representative mPPases of different clades based on their phylogenetic relationships and characterized them. We found that Na^+-PPases form a single clade on the phylogenetic tree, whereas H^+-PPases constitute multiple branches that diverged from Na^+-PPases on multiple occasions via subtle amino acid changes [3]. These data provide evidence that Na^+-PPases are an ancestral form of the transporter and support the theory that Na^+-based bioenergetics evolved before H^+-based bioenergetics. Furthermore, we discovered that Na^+-PPases are able to transport H^+ at subphysiological Na^+ concentrations [4] and identified a novel mPPase subfamily capable of transporting both Na^+ and H^+ at physiological Na^+ concentrations [5]. Functional and mutational analyses, together with structural information, allowed us to pinpoint Glu and Lys as a specificity-determining gate. Based on the available data, we created an algorithm to predict mPPase transport specificity from the amino acid sequence.

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doi:10.1016/j.bbabio.2014.05.268

S10.P14

Structural model of cytosolic N-terminus of a2-subunit isoform of V-ATPase: Identification and characterization of two binding interfaces for cytohesin-2

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The V-type ATPase (V-ATPase) is a multi-subunit membrane proton-pumping enzyme that is evolutionarily related to F- and A-type and ATP synthases [1,2]. In addition, it also serves as a pH-sensing and cytohesin-2 signaling receptor [3,4]. Here, we studied the molecular details of the interaction between the cytosolic N-terminus of the V-ATPase a2-subunit ($a2N_{1-402}$) and cytohesin-2 (CTH2). To generate a structural model of the complete $a2N_{1-402}$ we used a combination of *in silico* homology modeling and NMR data. First, using the crystal-structure of the bacterial *M. ruber* A-ATPase I_{cyt}-subunit as a template, we built a model of the mammalian $a2N_{1-352}$ fragment. Second, we determined the NMR structures of synthetic peptides $a2N_{368-395}$ and $a2N_{386-402}$ and combined them with our structural model of $a2N_{1-352}$. Third, we mapped the distribution of six V-ATPase-derived and CTH2-interacting peptides [5], which are clustered in two binding sites on $a2N_{1-402}$. Our data indicate that the proximal lobe sub-domain of $a2N_{1-402}$ ($a2N_{PL}$) is the major interacting site of the one CTH2 molecule (CTH2s) via its Sec7 domain, while the distal lobe of $a2N_{1-402}$ ($a2N_{DL}$) most likely interacts with the PH-domain of a second CTH2 molecule (CTH2p). This model was further tested using a Sec7/Arf-GEF activity assay. We confirmed that two Sec7-binding peptides $a2N_{1-17}$ and $a2N_{35-49}$ form an interaction interface on $a2N_{PL}$ and are involved in regulation of the enzymatic GEF activity of CTH2. Further analysis revealed that the binding sites of CTH2s and CTH2p are close to the binding sites of S1- and S2-EG-peripheral stalks of V-ATPase, located on $a2N_{PL}$ and $a2N_{DL}$ respectively [1]. We hypothesize that recruitment of CTH2 to V-ATPase during its function as a pH-sensing and CTH2-signaling receptor may also regulate assembly/disassembly and function of the V-ATPase. Thus, our study has revealed molecular details of the interactions between the V-ATPase and CTH2, which are important for regulation of their corresponding cell biological functions.

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doi:10.1016/j.bbabio.2014.05.269

S10.P15

Karlsberg+: A tool to predict pKa values and study proton transfer pathways in proteins using electrostatic energy calculations

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The software Karlsberg+ (http://agknapp.chemie.fu-berlin.de/karlsberg/) has been developed to be an easy to use tool for the prediction of pKa values in proteins using electrostatic energy calculations. A special feature of the software is the generation of pH adapted conformations. These are automatically modeled structures, representing the protein in a specific pH interval. The modeling includes relaxation of the hydrogen bond network, sampling of salt bridge geometries and in its latest version also a generalized approach that uses molecular dynamic simulations to sample structural conformations. This procedure has demonstrated to predict pKa values reliably [1,2]. The recent development of the software is focused on new features providing tools to analyze the detailed energetic and interaction of chargeable residues, based on the results of the electrostatic energy calculations. A special focus is set the identification and study proton transfer pathways in proteins.

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doi:10.1016/j.bbabio.2014.05.270

S10.P16

Mitochondrial iron regulation: Interaction of Mitoferrin-2 (Mfrn2) and the electrogenic mitochondrial calcium uniporter (MCU)

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MCU catalyzes electrogenic mitochondrial uptake of both Ca^{2+} and Fe^{2+} (JBC 1975;250:6433). More recently, Mfrn1 and 2 were identified to mediate mitochondrial iron uptake in erythroid and non-erythroid cells, respectively (Mol Cell Biol 2009;29:1007), whereas the CCDC109A gene product (MCU) was discovered to be the core protein of the uniporter complex (Nature 2011;476:336&341). Here, our aim was to determine the role of Mfrn2 in uniporter function. Respiration-driven Ru360-sensitive mitochondrial Fe^{2+} and Ca^{2+} uptake was measured in rat liver mitochondria (RLM) and permeabilized UMSCC1 and UMSCC22A head and neck squamous carcinoma cells. Overexpression and siRNA knockdown of Mfrn2 were performed in UMSCC1 and UMSCC22A cells, respectively. Pull down assays were performed in MCU-V5 and Mfrn2-GFP expressing HeLa cells. In RLM, Ca^{2+} and Fe^{2+} (250 μM) each stimulated respiration to a nearly identical degree, an effect that was completely blocked by Ru360. In UMSCC22A cells, mRNA and protein expression of Mfrn2 was 2–3-times higher than in UMSCC1 cells. High Mfrn2-expressing UMSCC22 cells had 3-fold greater rates of mitochondrial Ca^{2+} and Fe^{2+} uptake. After Mfrn2 knockdown (55% decrease), rates of mitochondrial uptake of both Ca^{2+} and Fe^{2+} decreased by 75%, whereas Mfrn2 overexpression increased Ca^{2+} and Fe^{2+} uptake by 56%. All uptakes were completely blocked by Ru360. In